Heritability of Brain Morphology Related to Schizophrenia: A Large-Scale Automated Magnetic Resonance Imaging Segmentation Study

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Background: Schizophrenia is a devastating psychiatric disorder with a strong genetic component that has been related to a number of structural brain alterations. Currently available data on the heritability of these structural changes are inconsistent.

Methods: To examine heritability of morphological alterations in a large sample, we used a novel and validated fully-automated whole brain segmentation technique to study disease-related variability and heritability in anatomically defined regions of interest in 221 healthy control subjects, 169 patients with schizophrenia, and 183 unaffected siblings.

Results: Compared with healthy control subjects, patients showed a bilateral decrease in hippocampal and cortical gray matter volume and increases in bilateral dorsal striatum and right lateral ventricle. No significant volumetric differences were found in unaffected siblings compared with normal control subjects in any structure. Post hoc analysis of the dorsal striatum showed the volumetric increase to be widespread, including caudate, putamen, and globus pallidus. With Risch's λ (λ_s), we found strong evidence for heritability of reduced cortical volume and moderate evidence for hippocampal volume, whereas abnormal striatal and ventricle volumes showed no sign of heritability. Additional exploratory analyses were performed on amygdala, thalamus, nucleus accumbens, ventral diencephalon, and cerebral and cerebellar cortex and white matter. Of these regions, patients showed increased volume in ventral diencephalon and cerebellum.

Conclusions: These findings support evidence of genetic control of brain volume even in adults, particularly of hippocampal and neocortical volume and of cortical volumetric reductions being familial, but do not support measures of subcortical volumes per se as representing intermediate biologic phenotypes.

Key Words: Brain volume, heritability, MRI, phenotypes, schizophrenia

Schizophrenia is a devastating and highly heritable psychiatric disorder that has been linked to numerous neurodevelopmental and morphological changes in the brain, including volumetric decreases in structures such as superior temporal gyrus, hippocampus, amygdala, thalamus, frontal lobe, and temporal lobe (1–3). These findings have been complemented by enlargements of the lateral and third ventricles as well as caudate, putamen, and globus pallidus (2–4).

The hippocampal formation is strongly implicated in schizophrenia (5), and a bilateral decrease in hippocampal volume has been among the most consistent results in volumetric studies of schizophrenia. One meta-analysis (2) reported a mean patient hippocampal volume of 98% and 97% (left and right) relative to normal control subjects. Furthermore, in 36 studies, an 81% rate of positive findings in the combined hippocampus and amygdala region was seen (3).

Of 20 studies, 70% showed volumetric increases in the basal ganglia (3). Overall volumetric increases have been found by meta-analyses in the caudate, putamen, and pallidum (2). Some have suggested that the increases in basal ganglia volume might be related to treatment with typical antipsychotic drugs, because volume did not increase in patients receiving atypical antipsychotic drugs (6), antipsychotic-naïve patients (7), and first-episode patients (8), and other studies have shown increased volumes under typical antipsychotic decrease when patients switched to second-generation antipsychotic drugs (9,10).

Because both genetic and environmental factors influence brain morphology in schizophrenia, the study of heritability of structural variations associated with schizophrenia offers a promising approach to further characterize the complex structural brain phenotype of this disorder. We pursued this strategy within the framework of the Clinical Brain Disorders Branch/National Institute of Mental Health (NIMH) sibling study (11), a large-scale study of patients with schizophrenia, unaffected siblings, and normal control subjects aimed at identifying schizophrenia susceptibility genes and related intermediate biologic phenotypes.

Three types of analyses were performed. First, we investigated whether changes observed in patients were also found in healthy siblings. This approach has been previously pursued in studies of brain cerebrospinal fluid, with inconsistent results (12,13), as well as for hippocampal volumes, which were found reduced in healthy relatives in some (14–16) but not all studies (17,18). In contrast to these structural markers, morphological changes in the dorsal striatum should not be heritable if they are primarily caused by treatment-related environmental factors, and at least one previous study (13) indeed did not find striatal abnormalities in first-degree relatives.

Although this type of analysis provides evidence for morphological phenotypes shared between patients and relatives, other sensitive and quantitative methods can be employed that interrogate variability attributable to genetic relatedness. We deter-

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mined two such parameters: the intraclass correlation coefficient (ICC), a measure of variability shared between pairs of siblings relative to variability across all probands; and Risch's λ for siblings (λ_s), a measure of presence of a categorical disease-related phenotype in healthy siblings of patients who exhibit that phenotype (19–21).

We analyzed a sample that included 221 normal control subjects, 169 patients with schizophrenia, and 183 unaffected siblings. On a largely overlapping dataset, our group has performed a set of optimized voxel-based morphometry (VBM) analyses (22), with methodology similar to that used previously to study regional volume change in patients with schizophrenia and their siblings (1,11). Because this study (22), based on voxel-based regional volume changes, which do not respect anatomical landmarks, shows only limited evidence for heritability, we applied here a complementary morphologically rigorous approach to delineate and estimate total volume for specific anatomical structures, hypothesizing that this would reveal clearer evidence of heritable abnormalities. To this end, anatomical magnetic resonance images (MRIs) were processed with a sophisticated and fully-automated segmentation algorithm that delineated gross morphology into gray matter (GM), white matter (WM), and a number of subcortical structures (23,24). Although the VBM method maps tissue class (GM, WM, cerebrospinal fluid) probability for each volume element (voxel) in an MRI scan to estimate regional volume or concentration, the segmentation method used in this study directly subdivides the image into a series of neuroanatomically defined structures with a priori knowledge of their individual intensity properties, atlas location, and location relative to each other. This approach provides advantages similar to manual region-of-interest (ROI) drawing, without the potential for rater bias, offering an anatomically accurate rendering of regional volumes not explicit in VBM approaches.

We hypothesized that our patient sample would show heritable reductions in cortical and hippocampal volume, compared with both normal control subjects and unaffected siblings. Furthermore, because the vast majority of the patients we studied were taking antipsychotic drugs, we hypothesized that patients with schizophrenia would exhibit a widespread increase in dorsal striatal volume, compared with both healthy control subjects and siblings. Also, we expected increased lateral and third ventricle volumes in patients compared with control subjects and unaffected siblings. We extracted and analyzed measures of local volume on the basis of these a priori hypotheses. Secondary exploratory post hoc analyses were performed on cerebral and cerebellar WM and cerebellar GM as well as on subcortical structures where prior data on volumetric changes were less compelling (e.g., amygdala, thalamus, ventral diencephalon, and nucleus accumbens).

Methods and Materials

Participants

Two hundred twenty-one healthy volunteers, 169 patients with schizophrenia, and 183 unaffected siblings of patients with schizophrenia were included in this study (Table 1). This sample largely overlapped with the one used in our VBM study (22) but was not identical, because the two methods involved separate processing streams and quality control subjects and a few images met quality criteria for only one method (see Imaging and Preprocessing section). Subjects were recruited nationwide in an ongoing family study of schizophrenia at the NIMH, Bethesda, Maryland, which included standard procedures such as a semi-structured diagnostic interview (Structured Clinical Interview for DSM-IV or SCID) (25) and formal neurological exam. Subjects provided written informed consent and participated according to the guidelines of the NIMH Institutional Review Board.

All patients met DSM-IV criteria for schizophrenia (78.7%), schizoaffective disorder (14.8%), or other related diagnoses including psychosis (not otherwise specified) and schizoid, schizotypal, and paranoid personality disorders (6.5%). The majority (89.1%) were receiving antipsychotic medication at the time of scan, and a subset (20.7%) had life-time history for comorbid substance abuse or dependence (including alcohol), depression, or bipolar disorder (Table 1), although there was no substance use for 6 months before evaluation. Because our affected patient group was broadly defined to include Cluster A personality disorders (schizoid, schizotypal, and paranoid), family members with these disorders were classified as affected

Table 1. Demographic Results

	Normal Volunteers	Affected Patients	Unaffected Siblings
Age	32.82 ± 9.51 (18.39–60.86)[a]	36.48 ± 10.13 (17.83–61.63)	36.38 ± 9.60 (17.37–58.25)
Gender	99 m, 122 f (45.0% m)[b]	130 m, 39 f (76.9% m)[b]	69 m, 114 f (37.7% m)[b]
WAIS Full-Scale IQ	107.11 ± 9.56 (78–129)	94.68 ± 12.00 (66–129)[a]	106.59 ± 10.57 (78–134)
Years of Education	16.82 ± 2.84 (12–25)[a]	14.25 ± 2.32 (9–23)[a]	16.10 ± 2.51 (10–27)[a]
Lifetime History of Mental Illness[c]	32 (14.5%)	35 (20.7%)[d]	66 (36.1%)
Bipolar Disorder[c]	0 (0%)	2 (1.2%)	0 (0%)
Major Depression[c]	23 (10.4%)	6 (3.8%)	49 (26.8%)
Personality Disorder[c]	5 (2.3%)	3 (1.8%)	8 (4.4%)
Substance Abuse/Dependence[c]	7 (3.2%)	30 (17.8%)	16 (8.7%)
Other Disorders[c]	5 (2.3%)	6 (3.5%)	22 (12.0%)
PANSS Positive Symptoms[e]	7.06 ± .31 (7–10)	11.91 ± 5.15 (7–27)[a]	7.06 ± .31 (7–9)
PANSS Negative Symptoms[e]	8.28 ± 2.87 (7–20)	18.19 ± 8.93 (7–44)[a]	8.23 ± 3.27 (7–28)
PANSS General Psychopathology[e]	16.36 ± 1.15 (16–24)	24.85 ± 8.55 (16–59)[a]	16.86 ± 2.14 (16–30)

PANSS, Positive and Negative Syndrome Scale; WAIS, Wechsler Adult Intelligence Scale.

[a]Demographic significantly differed from both other groups on the basis of simple contrasts.

[b]Gender significantly differed among the three groups on the basis of χ^2 tests.

[c]Some subjects have lifetime history for multiple illnesses. No participants exhibited current substance abuse, and no normal control subjects exhibited current Axis I disorders.

[d]Percentage does not include primary diagnosis of schizophrenia or related disorders.

[e]PANSS scores were available for 200 control subjects, 106 patients, and 158 siblings.

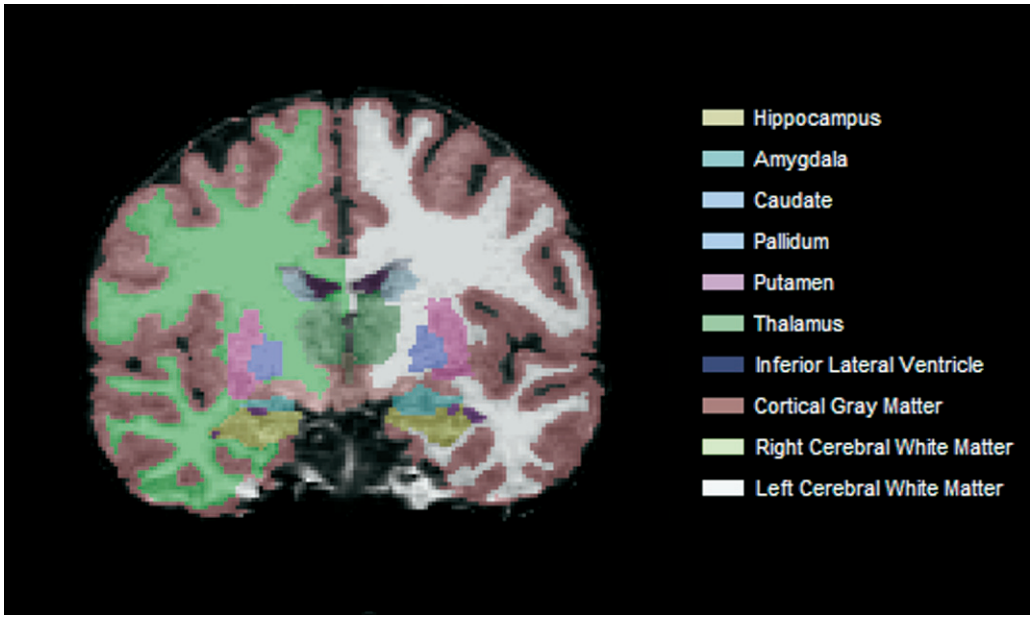

Figure 1. Sample segmented image: a coronal image showing typical automated subcortical segmentation results from Freesurfer. Different brain regions are indicated by different colors, for example hippocampus appears in gold, putamen in pink, and caudate and pallidum in two different shades of light blue. Total volumes are automatically extracted for each label, with separate values for left and right hemisphere in most structures.

patients and by definition absent from the unaffected sibling group. Supplemental analyses showed that this choice of criteria did not have a major impact on results (Supplement 1). No normal control subjects suffered from DSM-IV Axis I disorders at the time of scan, and no control subjects or siblings had current history of alcohol or substance abuse. However, a minority of control subjects (14.5%) and siblings (36.1%) had lifetime history of mental illness, including major depression, substance abuse or dependence, personality disorders, and other disorders (Table 1). Further diagnostic information can be found in Supplements 2 and 3.

Fifty-four pairs of one patient and one sibling were represented in the study along with 25 larger families with 3–5 members. Twenty-two families had 2–3 siblings or 2 patients

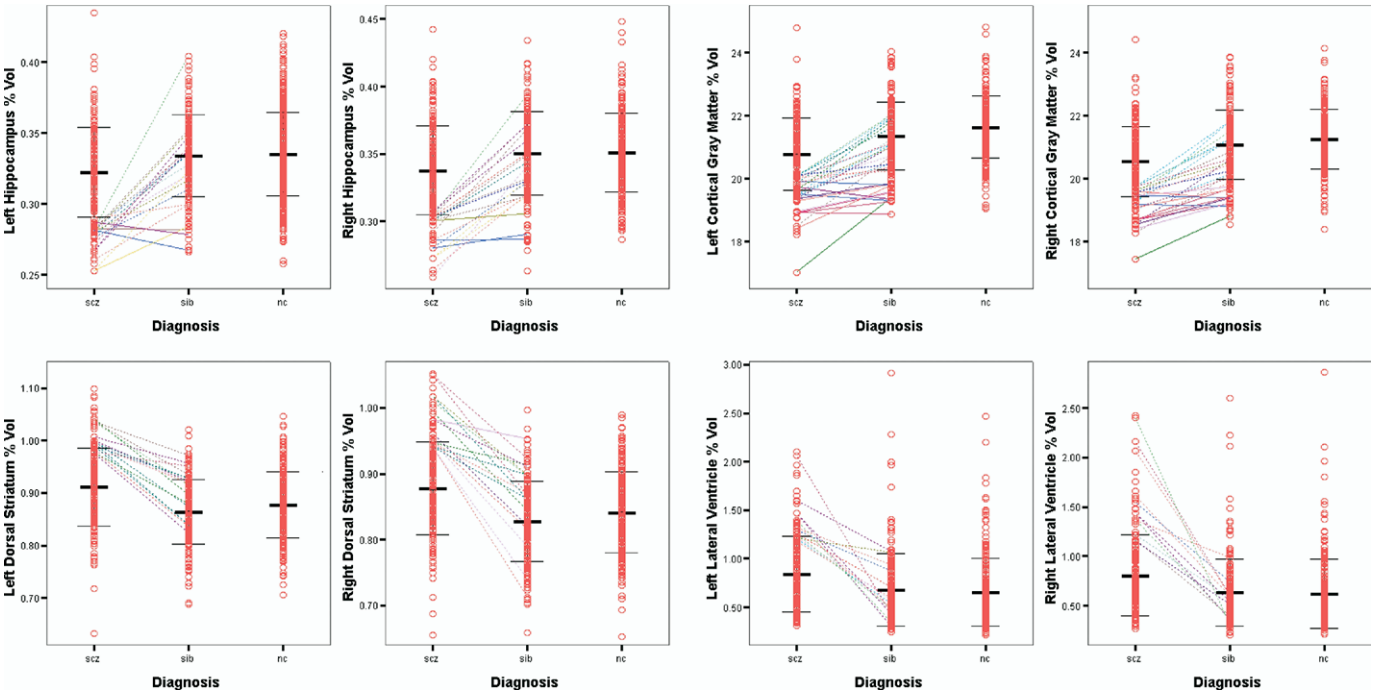

Figure 2. Volumetric distributions in hypothesis-driven regions of interest: red circles show the distribution of scaled volumes for subjects in each structure, and error bars show the mean and SD. Patients (scz) showed bilateral volumetric reductions in hippocampus and cortical gray matter (GM) compared with normal control subjects (nc) and bilateral increases in dorsal striatum and lateral ventricles compared with both control subjects and unaffected siblings (sib). Lines connect patients expressing each phenotype (i.e., having scaled volumes more than 1.5 SDs from the mean in the direction of our hypothesis) to their unaffected siblings. Solid lines represent cases where the unaffected sibling also expressed the phenotype, whereas dotted lines indicate cases where the siblings did not. According to Risch's λ tests (λ_s), siblings showed a relative risk for hippocampal and cortical GM reductions but not dorsal striatal or ventricle enlargement.

only, and the remainder of the patients (80) and siblings (51) did not have a relative included in this study.

Imaging and Preprocessing

Three-dimensional structural MRI scans were acquired on a 1.5-T GE scanner (Fairfield, Connecticut) with a T1-weighted spoiled gradient recalled sequence (repetition time/echo time/number of excitations 24/5/1, flip angle 45°, matrix size 256 × 256, field of view 24 × 24 cm), with 124 sagittal slices (0.94 × .94 × 1.5 mm resolution). Images were preprocessed and subcortically segmented (Figure 1) with Freesurfer's stable release 3.0.2 (23,24). Slices were resampled to a coronal three-dimensional image with 1-mm isotropic voxel size, followed by non-uniformity intensity normalization (N3) (26) and an affine transform registering images to Montreal Neurological Institute space (27). Next, a further intensity normalization step (with a different algorithm) was applied, in which WM control points were automatically identified and normalized to a standard intensity, followed by an automated skull strip (23,28).

Gross brain anatomy was then delineated into cortical and subcortical labels, with combined information on image intensity, probabilistic atlas location, and the local spatial relationships between subcortical structures (24,29). Careful visual quality checks were performed at several junctures of the processing stream, examining the Talairach-transformed, skull stripped, and segmented images of each subject, and failures were manually corrected and re-inspected. Corrections were performed on 53 patients and 28 control subjects and included manually realigning the Talairach template to subjects' images, setting intensity normalization control points in areas of stripped WM, and adjusting the skull strip's threshold. Finally, total volumes for each structure were extracted and scaled by dividing these values by the subject's "total label volume," which was the sum of all GM, WM, and ventricle labels (for absolute volumes, see Supplement 4). In Fischl *et al.* (24), inter-rater reliability between this method and manual segmentation was shown to be comparable to that between two manual raters. Additionally, we assessed test-retest reliability in a subset of our own affected patients, who showed over 98% agreement for cortical GM and over 96% agreement for hippocampus and dorsal striatum (see Supplement 5).

To ensure that manual edits did not confound our results, we analyzed the scaled volumes of affected patients in each structure, entering presence of manual edits and gender as fixed factors and age as a covariate in a general linear model (GLM). No significant effect of manual edits was found in any GM or WM label or with respect to lateral ventricle volume in either hemisphere. However, the third ventricle showed a significant effect [$F = 5.70$, $p = .018$], suggesting that automatic alignment fails more frequently in subjects with enlarged third ventricles, a pattern supported by patients showing the highest rate of Talairach transform failure.

Statistical Analysis

Final values derived from the subcortical segmentation process were then imported into SPSS 12.0 for statistical analysis (SPSS, Chicago, Illinois). In each hemisphere, the volumes of caudate, putamen, and pallidum were summed together to create one value for dorsal striatum, and total lateral ventricle volume was calculated by summing lateral and inferior lateral ventricle label volumes. Analyses were then performed on the volumes of third ventricle and left and right dorsal striatum, hippocampus, lateral ventricles, and cortical GM scaled by total label volume as detailed earlier. A univariate GLM tool was employed specifying diagnosis and gender entered as fixed factors and exact age-at-scan as a covariate. Post hoc simple contrasts for diagnosis were calculated to determine which groups significantly differed from each other. Additionally, Cohen's d and effect size (r) were calculated to assess the magnitude of the effects reported.

Two measures of heritability were performed for each region. First, ICC was used to measure the quantitative volumetric correlation within each sibling pair. Second, Risch's relative risk for siblings (λ_s) was calculated to quantitatively measure how likely siblings of patients with volumetric reductions or increases were to express the phenotype themselves. Because this parameter depends on the presence or absence of a phenotype, cut-offs were defined on the basis of the distribution of volumes in patients. For all subjects in each region, volumes were converted to Z scores on the basis of the mean and SD of normal control subjects, following the literature (19,20,30). In each structure, subjects were defined as expressing the hypothesized phenotype if their values were more than 1.5 SDs from the mean in the expected direction ($Z < -1.5$ for hippocampus, and $Z > 1.5$ for dorsal striatum and ventricles), corresponding to the outer 6.7% of the distribution. To calculate λ_s, we then compared the frequency of each phenotype in siblings of "patients with phenotype" with the frequency in normal control subjects. Absent familiarity, a trait is expected to have a λ_s value of 1, and relative risks of $\lambda_s > 2.0$ are considered evidence for familiality (31) and might reflect a heritable trait barring an effect of shared environmental factors. Because λ_s values were zero in some regions, post hoc results with a lower threshold ($|Z| > 1.0$) are provided as supplementary data.

To determine whether results in the dorsal striatum varied by subregion, post hoc analyses were also performed on the caudate, putamen, and pallidum individually in each hemisphere. Furthermore, because previous research on basal ganglia volume (6,8–10) demonstrated that antipsychotic use can influence regional volume, post hoc univariate GLMs were performed on our patient group for each main ROI, with antipsychotic use (on or off) and gender as fixed factors and age as a covariate. Finally, exploratory tests were performed on the remaining labels, which included bilateral amygdala, thalamus, ventral diencephalon, cerebral and cerebellar WM, and cerebellar cortex. A threshold of $p < .05$ was used for all analyses.

Results

Demographics

Table 1 shows demographic information separately for each diagnostic group, which were altogether representative of participants in this case-control study. To ensure there were no confounding age or gender interactions, preliminary GLM models were performed including gender-diagnosis and age-diagnosis interactions. No significant interactions were found in dorsal striatum, cortical GM, or hippocampus, although age-diagnosis was significant in the left lateral ($p = .021$) and third ($p < .002$) ventricles, with diagnosis effects becoming more pronounced with age (see Lateral and Third Ventricles). Consequently, age and gender were included in all subsequent analyses as confounding covariates as well as interaction terms for those structures in which they were significant.

Hippocampus

Diagnosis (group membership) significantly affected bilateral hippocampal volume [left $F = 4.01$, $p = .019$; right $F = 3.13$, $p = .044$; Table 2], with patients showing a significant reduction

Table 2. Volumetric Results in Hypothesis-Driven Regions of Interest

	Diagnosis		Gender		Age		Diagnosis × Age[a]	
Region	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Left Hippocampus	4.01	.019*	40.64	3.8×10^{-10}*	7.59	.0060*	—	—
Right Hippocampus	3.13	.044*	47.41	1.5×10^{-11}*	2.63	.11	—	—
Left Dorsal Striatum	25.88	1.8×10^{-11}*	3.56	.060	13.06	.00033*	—	—
Right Dorsal Striatum	28.02	2.5×10^{-12}*	1.11	.29	7.32	.0070*	—	—
Left Lateral Ventricle	1.54	.22	1.75	.19	62.54	1.4×10^{-14}*	4.29	.014*
Right Lateral Ventricle	9.28	.00011*	3.66	.056	59.77	4.9×10^{-14}*	—	—
Third Ventricle	1.72	.18	.16	.69	59.43	5.7×10^{-14}*	5.98	.0027*
Left Cerebral Cortex	16.40	1.2×10^{-7}*	31.74	2.8×10^{-8}*	377.20	8.1×10^{-65}*	—	—
Right Cerebral Cortex	11.66	1.1×10^{-5}*	24.67	9.0×10^{-7}*	353.11	1.3×10^{-61}*	—	—

* Significant at $p < .05$ level. Volumes of each structure, scaled to total label volume, were analyzed in a univariate general linear model, with diagnosis and gender as fixed factors and age as a covariate.

[a]Diagnosis × age interactions were included in models only when preliminary tests showed them to be significant. Diagnosis × gender is not shown, because it was not significant in any preliminary tests.

compared with control subjects (left $p < .005$; right $p = .013$), whereas siblings did not significantly differ from patients or control subjects (Table 3). The ICC tests detected only a weak correlation of hippocampal volumes between patient-sibling pairs; however, Risch's λ indicated heritability by showing increased relative risk for reduced hippocampal volume (left $\lambda_s = 3.10$; right $\lambda_s = 2.33$; Table 4, Figure 2).

Dorsal Striatum

Likewise, we found a significant impact of diagnosis on bilateral dorsal striatum volume [left $F = 25.88$, $p < .001$; right $F = 28.02$, $p < .001$; Table 2], with patients showing increased dorsal striatum volume compared with both other groups ($p < .001$), who did not differ from each other (Table 3). The ICC tests showed little correlation of dorsal striatal volume between

Table 3. Post Hoc Contrasts in Hypotheis-Driven Regions of Interest

	Patients vs. Controls			Patients vs. Siblings			Siblings vs. Controls[a]		
Region	*p*	*d*	*r*	*p*	*d*	*r*	*p*	*d*	*r*
Left Hippocampus	.0048*	.42	.20	.095	.39	.19	.25	.034	.017
Right Hippocampus	.013*	.42	.20	.10	.39	.19	.41	.013	.0066
Left Dorsal Striatum	1.8×10^{-8}*	.48	.23	1.8×10^{-11}*	.68	.32	.13	−.21	−.10
Right Dorsal Striatum	9.8×10^{-9}*	.53	.26	1.9×10^{-12}*	.74	.35	.074	−.23	−.11
Left Lateral Ventricle	.50	.55	.27	.085	.44	.21	.23	.10	.050
Right Lateral Ventricle	.00033*	.52	.25	7.2×10^{-5}*	.48	.23	.56	.054	.027
Third Ventricle	.55	.54	.26	.073	.56	.27	.17	−.0089	−.01
Left Cerebral Cortex	1.0×10^{-7}*	.81	.37	3.6×10^{-6}*	.52	.25	.58	.27	.13
Right Cerebral Cortex	5.5×10^{-5}*	.72	.34	1.1×10^{-5}*	.49	.24	.60	.18	.090

* Significant at $p < .05$ level. Post hoc simple contrasts were performed for diagnosis in each structure, and Cohen's *d* and effect size (*r*) were calculated to assess the magnitude of effects.

[a]Negative *d* and *r* values indicate effects in the opposite direction of patients vs. controls.

Table 4. Intraclass Correlation and λ_s in Regions of Interest

	ICC			λ_s[a]		
Region	ICC	95% CI Lower	95% CI Upper	Siblings with Phenotype[b]	Controls with Phenotype	λ_s
Left Hippocampus	.12	−.15	.37	4/19	15/221	3.10
Right Hippocampus	.23	−.038	.47	3/19	15/221	2.33
Left Dorsal Striatum	.30	.038	.52	0/15	16/221	0
Right Dorsal Striatum	.33	.067	.54	1/15	17/221	.87
Left Lateral Ventricle	.091	−.19	.36	0/17	15/221	0
Right Lateral Ventricle	.0078	−.27	.28	0/15	16/221	0
Left Cerebral Cortex	.62	.41	.77	11/29	17/221	4.93
Right Cerebral Cortex	.62	.41	.77	11/26	11/221	8.50

Intraclass correlation and Risch's λ (λ_s) were calculated as measures of heritability. For Risch's λ, $\lambda_s = 1$ is the expected value for non-familial traits, whereas $\lambda_s > 2.0$ is considered evidence for familiality.

CI, confidence interval; ICC, intraclass correlation coefficient.

[a]Risch's λ measurements were taken with a threshold of $|Z| > 1.5$.

[b]For each region, Risch's λ analysis includes only siblings of patients beyond the threshold. Thus the denominator for siblings differed in each region (see Statistical Analysis section).

Table 5. Post Hoc Exploratory Volumetric Results

Region	Diagnosis		Gender		Age		Diagnosis × Gender[a]		Diagnosis × Age[a]	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Left Amygdala	2.47	.086	.42	.52	5.93	.015*	—	—	—	—
Right Amygdala	.41	.66	.088	.77	6.80	.0093*	—	—	—	—
Left Thalamus	4.25	.015*	14.10	.00019*	21.86	3.7×10^{-6}*	—	—	5.40	.0048*
Right Thalamus	.23	.80	8.73	.0033*	5.30	.022 *	—	—	—	—
Left Ncl. Accumbens	.92	.40	5.53	.019*	7.97	.0049*	—	—	—	—
Right Ncl. Accumbens	2.16	.12	4.75	.030*	19.43	1.3×10^{-5}*	—	—	—	—
Left Ventral Diencephalon	3.56	.029*	2.07	.15	.14	.71	—	—	—	—
Right Ventral Diencephalon	6.99	.0010*	1.42	.24	1.53	.22	—	—	—	—
Left Cerebral WM	.51	.60	31.87	2.6×10^{-8}*	216.73	8.6×10^{-42}*	—	—	—	—
Right Cerebral WM	.38	.69	17.80	2.9×10^{-5}*	176.38	3.0×10^{-35}*	—	—	—	—
Left Cerebellar GM	2.67	.070	.91	.34	.17	.68	5.61	.0039*	—	—
Right Cerebellar GM	2.00	.14	.067	.80	.33	.57	5.68	.0036*	—	—
Left Cerebellar WM	.39	.68	7.35	.0069*	17.61	3.2×10^{-5}*	—	—	—	—
Right Cerebellar WM	3.49	.031*	1.23	.27	40.45	4.2×10^{-10}*	7.10	.0012*	5.30	.0052*

* Significant at $p < .05$ level. Volumes of each structure, scaled to total label volume, were analyzed in a univariate general linear model, with diagnosis and gender as fixed factors and age as a covariate.

GM, gray matter; Ncl., nucleus; WM, white matter.

[a]Diagnosis × age and diagnosis × gender interactions were included in models only when preliminary tests showed them to be significant.

patient-sibling pairs (Table 4), and no relative risk was found (left $\lambda_s = 0$; right $\lambda_s = .87$; Table 4, Figure 1). Supplemental Risch's λ tests with a lowered threshold ($Z > 1$) also provided no evidence for heritability (left $\lambda_s = .94$; right $\lambda_s = .89$; Supplement 6).

Post hoc analyses of the caudate, putamen, and pallidum revealed similar significant bilateral main effects in all three regions, indicating widespread dorsal striatum enlargements (Supplement 7). Finally, tests for effects of antipsychotic use in the patient group for our ROIs revealed significant effects exclusively in the left dorsal striatum [$F = 4.06$, $p = .046$], with patients who were taking antipsychotic drugs showing increased volume (Supplement 8).

Lateral and Third Ventricles

A significant main effect of diagnosis was found in right lateral ventricle [$F = 9.28$, $p < .001$], which was significantly enlarged in patients compared with both normal control subjects and siblings ($p < .001$), who did not differ from each other (Table 3). A diagnosis × age interaction was included in the model for left lateral and third ventricles, as described in the Methods section, because preliminary tests revealed the interaction to be significant. These remained significant in the final model for both structures [left lateral $F = 4.29$, $p = .014$; third $F = 5.98$, $p < .003$], but no main effects of diagnosis were found (Table 2). Post hoc examination of these two structures revealed a general trend toward increased ventricular volume in patients, which became more pronounced with age (Supplement 9).

The ICC measurements showed only a weak correlation among sibling-patient pairs (Table 4), and λ_s was zero for both lateral ventricles (Table 4, Figure 1). Supplemental Risch's λ tests with a lowered threshold ($Z > 1$) produced a λ_s of 1.49 for left lateral ventricle, and .64 for right (Supplements 6 and 10).

Cortical GM

A highly significant main effect of diagnosis was seen bilaterally in cerebral cortex [left $F = 16.40$, $p < .001$; right $F = 11.66$, $p < .001$], with patients showing significant reductions compared with both control subjects and siblings ($p < .001$), who did not differ from each other (Table 3).The ICC tests showed a strong correlation in cortical GM volume between patient-sibling pairs (Table 4), and Risch's λ tests showed strong evidence for heritability by indicating increased relative risk for reduced cortical GM volume (left $\lambda_s = 4.93$; right $\lambda_s = 8.50$; Table 4, Figure 1).

Other Regions

Finally, post hoc exploratory analyses were performed for amygdala, thalamus, nucleus accumbens, ventral diencephalon, cerebral WM, and cerebellar GM and WM (Table 5). As before, age × diagnosis and age × gender interactions were included when significant in preliminary tests. Thus, the model for left thalamus included diagnosis × age, cerebellar GM bilaterally included diagnosis × gender, and right cerebellar WM contained both interactions.

Further diagnosis effects and/or interactions were seen in ventral diencephalon, cerebellar GM, right cerebellar WM, and left thalamus (Table 5). Specifically, patients showed increased volume in ventral diencephalon and, in the case of men, cerebellar GM and right cerebellar WM. In the left thalamus, siblings seemed to show less relative volumetric decrease with age (Supplement 11). No diagnosis effect or interactions were seen in amygdala, right thalamus, nucleus accumbens, cerebral WM, or left cerebellar WM (Table 5).

Discussion

The present study assessed volume changes and heritability of brain morphological phenotypes associated with schizophrenia with a large sample of patients with schizophrenia, unaffected siblings, and healthy volunteers. A sophisticated, fully-automated method was used to process structural brain images and divide them into a number of cortical and subcortical structures. The results provide confirmatory evidence for several widely reported morphological phenotypes in schizophrenia, demonstrate the feasibility of automated subcortical segmentation as a high-throughput neuroscience technique for the analysis of large structural datasets in schizophrenia, and help clarify the role of heritability for the studied phenotypes.

As predicted (2,3,32), our sample of patients with schizophrenia showed a bilateral decrease in hippocampal volume. We found no similar reduction in unaffected siblings, replicating previous research (17,18) but contrary to some other studies (14–16). However, the moderate relative risk we observed for hippocampal reductions suggests a heritable component, in accordance with reports implicating risk genes for schizophrenia in hippocampal volume (5), indicating that reductions in regional volume in siblings are not a necessary condition for a heritable intermediate phenotype and might in fact be less sensitive than tests for variability attributable to genetic relatedness. Our results corroborate our previous observation (30) that Risch's λ was superior to ICC in detecting familiality for this trait. This is expected, because ICC is driven by overall genetic influences on a given structure, whereas Risch's λ specifically tests for the presence of a disease-related phenotype (reduction in volume), making it more sensitive to disease-related genetic variation (i.e., genetic susceptibility) if the phenotype under study is in fact impacted by risk genes for schizophrenia. However, Risch's λ, as derived, assumes that the phenotype is a discrete, ordinal trait, and brain morphology is quantitative, with variance attributable to multiple additive/interacting genes.

Patients also showed a significant volumetric increase of all structures forming the dorsal striatum, consistent with previous research (2–4,33). Importantly, despite being highly significant, these volumetric increases showed no evidence of heritability. Unaffected siblings did not show increased striatal volume, and no evidence for heritability was found with Risch's λ and ICC. Additionally, post hoc tests for medication effects in our main ROIs revealed a significant enlargement in left dorsal striatum associated with medication use. Thus, the results of this study are consistent with and further support data associating dorsal striatum enlargements with typical antipsychotic drug use (6,8–10).

As expected, our results corroborate the consistently observed ventricular enlargement associated with schizophrenia. The fact that significant ventricle enlargement was found in only one compartment and absent evidence for heritability indicates that this structural feature, although often conspicuous, might be relatively unspecific and not directly linked to the core neurobiology of the disorder. The lack of observed heritability for ventricular enlargements in this study is consistent with previous heritability studies, which link ventricular volume (12,34) or shape (35) to environmental or general genetic factors but not to genetic risk factors related to schizophrenia, although differing conclusions have been found by others (17,36). Significant age × diagnosis interactions in this cross-sectional study are unlikely to reflect an intermediate phenotype close to the core of the disorder—because no evidence of heritability was found—and are also highly unlikely to reflect a degenerative component to the disease (37). Postmortem studies have found no consistent evidence, such as cortical neuron loss, gliosis, or neuronal degeneration, that would support a degenerative model (38), and cognitive studies have not generally suggested a progressive loss of function (39). Rather than degeneration, interactive effects of diagnosis with age might reflect neuroplastic changes in response to factors such as lack of stimulation, chronic medication use, smoking, or substance use (37).

Finally, a highly significant overall reduction in cortical GM was seen in patients compared with control subjects and unaffected siblings, supporting the idea of widespread GM reductions associated with schizophrenia. The categorical phenotype of reduced cortical GM showed a strong heritable component, according to both Risch's λ and ICC measures. Whereas cortical volume has been shown to be a highly heritable characteristic in general (40), twin studies have been important in specifically linking cortical reductions to genetic risk for schizophrenia (41,42). By comparing monozygotic twins discordant to schizophrenia with healthy twin pairs and dizygotic discordant twin pairs, these studies as well as the present data provide evidence for heritable, non-environmental reductions related to schizophrenia (41). Previous research has implicated a number of specific areas of cortical GM, most notably prefrontal cortex and superior temporal gyrus, that show the most compelling reductions (2,3). However, the segmentation algorithm was not designed to parcellate specific cortical GM areas. Complementary methods such as VBM are useful to identify cortical subregions especially affected in schizophrenia (1,22,33). In addition, surface-based methods reliant on anatomical landmarks defined by gyrification can be used (43).

The automated method used in this study allows the advantages of a ROI-based approach with a sample size much larger than would normally be practical and is especially suitable for analyzing small subcortical structures such as the hippocampus. Further work can now use the delineated subcortical structure to measure structural parameters beyond regional or voxel-wise volume, such as shape (35). Furthermore, including a large sample of unaffected siblings in our design enabled us to test for the heritability of the quantitative intermediate phenotypes we measured. These results revealed a marked distinction between the heritability of hippocampal and cortical reductions and the lack of heritability for volumetric increases in dorsal striatum and lateral ventricles, analogous to findings in previous studies of monozygotic twins (34,41). Solid effect sizes were found in all of our hypothesis-driven results, demonstrating that diagnosis produced substantial volumetric differences in these structures.

Despite the automated nature of this method, it should be noted that manual interventions were necessary, because a minority of the sample (81 subjects) required manual correction of the registration or skull strip. However, as mentioned in the Imaging and Preprocessing section, this was not linked to significant volume differences in any structure except third ventricle, suggesting that manual intervention was not a major confound. It seems possible that difficulties with aligning scans of subjects with ventricular enlargements might have contributed to greater variability of this measurement, although no positive evidence for this was apparent in the data. Although MRI-based morphometry is a powerful tool, it should be noted that results can be affected by movement and other acquisition-related sources of variation (44) and do not directly reflect the amount of neural tissue being affected by confounding factors such as hydration status, cerebral perfusion, and possibly smoking. However, the combination of multimodal imaging (both structural and functional) with genetic and post-mortem approaches is expected to provide convergent evidence for the biological basis of observed morphometric differences (37,38).

Conclusions

The results of this study support several commonly reported volumetric differences associated with schizophrenia, including reductions in hippocampal and total cortical GM volume, ventricular enlargement, and widespread volumetric increases in dorsal striatum. Although no volumetric differ-

ences were observed between unaffected siblings and normal control subjects in these regions, evidence for heritability of reduced GM volume as a schizophrenia-associated biologic trait was found in both hippocampus and cortical GM. Lack of heritability in the dorsal striatum supports the notion that enlargements in this region are primarily an effect of antipsychotic treatment.

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Supplementary material cited in this article is available online.

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